ab197003
Dihydroxyacetone Phosphate (DHAP) Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Dihydroxyacetone Phosphate (DHAP) in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 4a, Last Updated 5 June 2025

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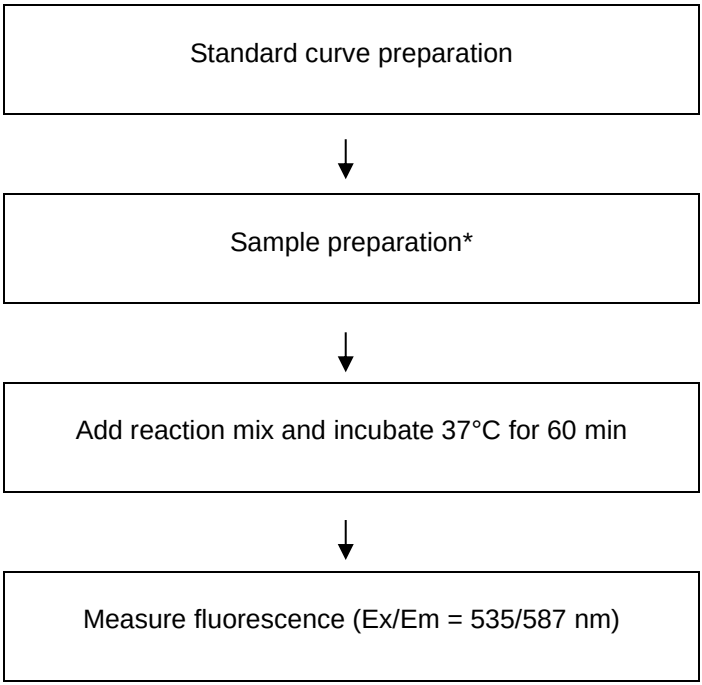
1. BACKGROUND

Dihydroxyacetone Phosphate (DHAP) Assay kit (Fluorometric) (ab197003) is suitable for measuring low levels of DHAP typically found in a variety of samples. In this kit, TPI converts DHAP to GAP that undergoes a series of reaction and reduces PicoProbe to generate fluorescence. The fluorescence intensity generated is directly proportional to the amount of dihydroxyacetone phosphate. This simple, sensitive, and easy to use assay kit can detect Dihydroxyacetone Phosphate as low as 0.5 µM in a variety of samples.

Dihydroxyacetone Phosphate (DHAP) is an important intermediate in both lipid biosynthesis and glycolysis. In glycolysis, fructose-1,6-diphosphate is converted to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP) by aldolase. Both DHAP and GAP serve as the intracellular triose phosphate pool. DHAP can be further converted into GAP by Triose Phosphate Isomerase (TPI). In humans, TPI deficiency is a rare autosomal disease. It causes hemolytic anemia, neurological diseases, and even death due to blockage of the glycolytic pathway and accumulation of DHAP in erythrocytes.

2. ASSAY SUMMARY



*Samples might require deproteinization.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 2	25 mL	-20°C	-20°C
PicoProbe I	0.4 mL	-20°C	-20°C
DHAP Enzyme Mix	1 vial	-20°C	-20°C
Developer Solution X	1 vial	-20°C	-20°C
DHAP Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and DHAP Assay Buffer. The composition has not changed

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em =535/587 nm
- 96 well plate: white flat bottom plates for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

For deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 2:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **PicoProbe I:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light and moisture. Once the probe is thawed, use with two months.

9.3 **DHAP Enzyme Mix:**

Reconstitute in 220 µL Assay Buffer 2. Ensure the material is completely dissolved. Aliquot enzyme so that you have enough volume to perform the desired number of tests. Avoid repeated freeze/thaw cycles. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **Developer Solution X:**

Reconstitute in 220 µL Assay Buffer 2. Ensure the material is completely dissolved. Aliquot developer so that you have enough volume to perform the desired number of tests. Avoid repeated freeze/thaw cycles. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **DHAP Standard:**

Reconstitute the in 100 µL of ddH_2O to generate a 100 mM (100 nmol/µL) standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 1 mM DHAP standard by diluting 10 µL of the reconstituted 100 mM DHAP standard with 990 µL of Assay Buffer 2.

10.2 Prepare 50 µM (50 pmol/µL) DHAP Standard by diluting 50 µL of 1 mM DHAP standard with 950 µL of Assay Buffer 2.

10.3 Using 50 µM (50 pmol/µL) DHAP standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. DHAP in well
1	0	150	50	0 pmol/well
2	6	144	50	100 pmol/well
3	12	138	50	200 pmol/well
4	18	132	50	300 pmol/well
5	24	126	50	400 pmol/well
6	30	120	50	500 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the Deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer 2.

11.1.4 Homogenize cells with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.1.5 Keep on ice for 10 minutes.

11.1.6 Centrifuge sample for 5 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.1.8 Keep on ice.

11.1.9 Perform deproteinization step as described in section 11.4.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer 2.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Keep on ice for 10 minutes.

11.2.6 Centrifuge samples for 5 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.

11.2.7 Collect supernatant and transfer to a clean tube.

11.2.8 Keep on ice.

11.2.9 Perform deproteinization step as described in section 11.4.

11.3 **Plasma, Serum and Urine and other biological fluids:**

Plasma, serum and urine samples generally contain high amount of proteins, so they should be deproteinized as described in section 11.4.

Alternatively, you can use 10kD Spin column (ab93349) to deproteinize biological fluids.

Serum and urine samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

11.4 **Deproteinization step:**

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.4.1 Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE****: high protein concentration samples might need more PCA.*

11.4.2 Incubate on ice for 5 minutes.

11.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.4.4 Precipitate excess PCA by adding an equal volume of ice-cold 2 M KOH to supernatant obtained in previous step and

vortex briefly. This will neutralize the sample and precipitate excess PCA. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). Any left over PCA will interfere with the assay.

11.4.5 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **NADH in samples will generate background. For samples containing NADH, prepare parallel sample well(s) as sample background control(s).**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample wells= 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). ***NOTE:*** *for samples containing NADH, as they can generate high background.*

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 2	**43**	**45**
PicoProbe I	**3**	**3**
DHAP Enzyme Mix	**2**	**0**
Developer Solution X	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards +1).

12.3 Add 50 µL of Reaction Mix into each standard and sample wells.

12.4 Add 50 µL of Background control mix into each background control sample well.

12.5 Mix and incubate at 37°C for 60 minutes protected from light.

12.6 Measure fluorescence at Ex/Em = 535/587 nm on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of DHAP.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of DHAP (pmol/µL or nmol/mL) in the test samples is calculated as:

$$DHAP\ concentration = \left(\frac{B}{V}\right) * D$$

Where:

B = Amount of DHAP in the sample well pmol.

V = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Dihydroxyacetone Phosphate ($C_3H_7O_6P$) molecular weight: 170.06 g/mol.

Dihydroxyacetone Phosphate in sample can also be expressed in pmol/mg or mg/dL of sample.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

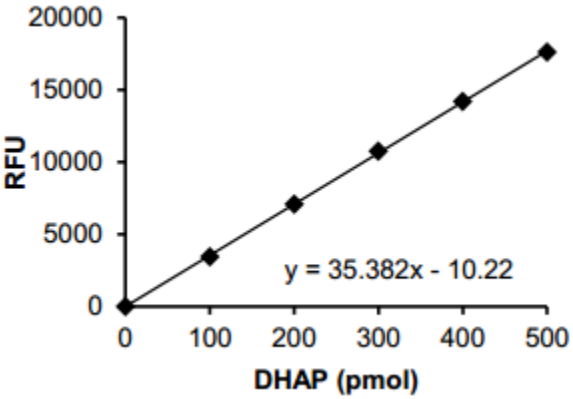


Figure 1. Typical DHAP Standard calibration curve using fluorometric reading.

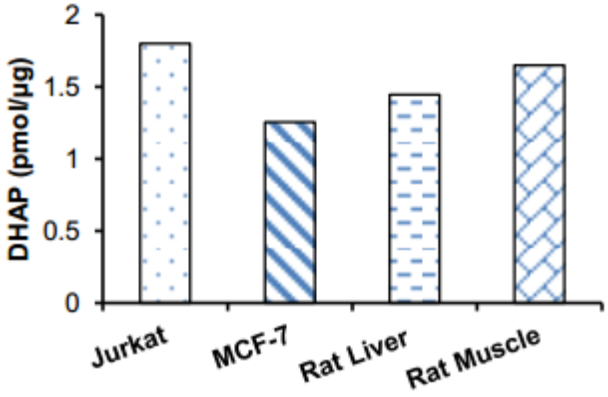


Figure 2: Measurement of Dihydroxyacetone Phosphate level in Jurkat (250 µg), and MCF-7 (150 µg) cell lysate, and in rat liver (50 µg) and muscle (150 µg) lysate.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare assay buffer, probe, enzyme mix, developer and standard; (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings), including deproteinization step.
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare DHAP Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 2	**43**	**45**
PicoProbe I	**3**	**3**
DHAP Enzyme Mix	**2**	**0**
Developer Solution X	**2**	**2**

- Add 50 µL of DHAP Reaction Mix to the standard and sample wells.
- Add 50 µL of DHAP Background control mix to the standard and sample wells.
- Incubate plate at 37°C 60 min protected from light.
- Measure plate at Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. **FAQ**

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Enzymes in sample – deproteinize sample following PCA protocol or using 10kD Spin Column (ab93349)

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)